secrete chloride in response to cAMP stimulation. Therefore, there is an inadequate amount of water on the luminal side of the epithelial membranes as well as excessive sodium reabsorption. In airway cells this causes abnormal mucus secretion with inadequate water content, ultimately leading to pulmonary infection and epithelial damage. Abnormal electrolytes in the sweat of CF patients probably results from the impermeability of the sweat duct epithelium to chloride.

Physiologically, the (ΔF508) mutant CFTR is mis-folded and unable to assume its appropriate tertiary conformation (Thomas *et al.*, J. Biol. Chem. 267:5727-5730 (1992)), is retained in the endoplasmic reticulum (ER) as a result of the mutation-induced mis-folding, and eventually is targeted for degradation (Cheng *et al.*, Cell 63:827-834 (1990); Ward *et al.*, Cell 83: 122-127 (1995)). Other examples of processing mutants leading to CFTR chloride channel dysfunction, with the frequency of the mutation in parentheses, include: DI507 (0.5), S549I (very rare), S549R (0.3), A559T (very rare) and N1303K (1.8) (Welsh *et al.*, Cell 73:1251-1254 (1993)). P574H and A455E are additional CF-associated mutants which are also mis-processed (Ostedgaard *et al.*, J. Cell. Sci. 112(Pt13):2091-2098 (1999)). Only 5% to 10% of the mis-folded CFTR protein of these two mutants reaches the apical membrane.

Because more than 98% of CF patients die from either respiratory failure or pulmonary complications before reaching maximum physiological maturity, the therapeutic goals have historically been to prevent and treat the complications of obstruction and infection in the airways, enhance mucous clearance, and improve nutrition. The identification of the ΔF508 defect (and other mutations in CFTR) has facilitated the rapid development of proposed treatments for CF, including the therapeutic introduction of the wild-type CFTR gene via gene therapy, as well as more traditional drug therapies.

**C. Current and Potential Treatments for Cystic Fibrosis**

**Treatment of Cystic Fibrosis Using Traditional Drugs.** Traditional treatments for CF include chest physiotherapy (*e.g.*, percussion and postural drainage), various broncodilators, nutritional supplements (*e.g.*, pancreatic enzymes and vitamins), exercise and rehabilitation, and long-term oxygen therapy for chronic hypoxemia. Aerosolized amiloride has been administered to improve the quality of the secretions, thereby improving the air flow in CF patients (U.S. Patent Nos. 4,501,729 and 4,866,072). Although these methods have increased the overall survival and physical comfort of CF patients, the traditional drugs and treatment methodologies do not cure the afflicted

individuals and CF-afflicted persons often are not expected to live beyond their mid-twenties or early thirties. (R.C. Bone, *supra*).

**DNase Treatment.** One identified new drug treatment for CF has been the use of DNase, such as human DNase 1, which ameliorates one of the side effects caused by the defect in CFTR (New England Journal of Medicine 331:637-642 (1994)). Although the water content of bronchial secretions is probably the critical determinant of secretion viscosity, it is believed that DNA from lysed cells may add to this index.

**Increased Permeability of Epithelial Cells to $Cl^-$.** U.S. Patent No. 5,384,128 discloses a method of treating CF which comprises administration of an epithelial cell chloride permeability enhancing composition which is a nontoxic, nonionic surfactant having (1) a critical micelle concentration of less than about 10 mM and a hydrophile-lipophile balance number of from about 10 to 20, and (2) a suitable hydrophobic organic group joined by a linkage to a suitable hydrophobic polyol. Examples of such compositions include a saccharide joined with organic groupings, such as an alkyl, aryl, aralkyl, or fatty acid group; polyoxyethylenes joined with an organic grouping; or, alkyl polyoxyethylene sorbitans. The preferred method of treatment is by aerosol inhalation.

**Treatment of Cystic Fibrosis Using Gene Therapy.** Several methods of gene therapy have been developed and are being tested for providing the normal CFTR gene into CF patients. For example, transfecting the normal CFTR gene into the nasal epithelial cells of patients has been shown to improve functions of the transmembrane chloride channel. These results have raised the hope that delivery of retroviral vectors containing normal CFTR genes directly to the lung epithelium by means of aerosol will help alleviate CF. Despite promising results, implementation of gene therapy methodologies to "cure" CF still remain in the experimental stages. As a result, an efficacious drug alternative to proposed gene therapy treatments is needed to more effectively treat CF.

**D. Chronic Obstructive Pulmonary Disease: An Overview of the Disease, Protein and Gene.**

**The Disease.** The designation Chronic Obstructive Pulmonary Disease (COPD) is an imperfect, although widely used, term because it includes several specific disorders with different clinical manifestations, pathologic findings, therapy requirements, and prognoses. The term encompasses chronic bronchitis and emphysema. Common to most of these diseases is chronic involvement of peripheral (small) airways or, more rarely, localized obstruction of central (large) airways. For a comprehensive overview of COPD, see

Matthay *et al.*, Chronic Airways Diseases, *In* Cecil Textbook of Medicine (Bennet *et al.*, eds.; W. B. Saunders Company) 20th Ed., 52:381-309 (1996)).

Since elastase released by activated neutrophils is rendered inactive by the inhibitor α-antitrypsin (AAT), diminished circulating levels of AAT can result in proteolytic destruction of lung elastin, a phenomenon implicated in the pathogenesis of COPD (Travis *et al.*, Annu. Rev. Biochem. 52:655-709 (1983); Beith, Front. Matrix Biol. 6:1-4 (1978)).

**The α-Antitrypsin (AAT) Protein and Gene.** Human AAT is a 394-amino acid protein glycosylated at three specific asparagine residues (Carrell *et al.*, *In* Proteinase Inhibitors (Barrett *et al.*, eds.; Elsevier, Amsterdam) 403-420 (1986); Long *et al.*, Biochemistry 23:4828-4837 (1984); Yoshida *et al.*, Arch. Biochem. Biophys. 195:591-595 (1979)). AAT is a member of the serine proteinase inhibitor superfamily (Huber *et al.*, Biochemistry 28:8951-8966 (1989)). It is folded into a highly ordered tertiary structure containing three β-sheets, nine α helices, and three internal salt bridges (Loebermann *et al.*, J. Mol. Bio. 177:531-556 (1984)).

**Gene Mutations Responsible for COPD.** The human AAT structural gene is highly polymorphic and several alleles exhibit a distinct mutation predicted to preclude conformational maturation of the encoded polypeptide following biosynthesis (Brantly *et al.*, *Am. J. Med.* 84:13-31 (1988); Stein *et al.*, *Nat. Struct. Biol.* 2:96-113 (1995)). Genetic variants of human AAT unable to fold into the native structural conformation are poorly secreted from hepatocytes (Laurell *et al.*, *In* Protease Inhibitors in Plasma (Putnam, ed.; Academic Press, New York) Vol. 1:229-264 (1975); Peters *et al.*, *In* Plasma Protein Secretion by the Liver (Glaumann *et al.*, eds.; Academic Press, New York) 1-5 (1983); Sifers *et al.*, Semin. Liver Dis. 12:301-312 (1992); Sifers *et al.*, *In* The Liver: Biology and Pathology (Arias et al., eds.; Raven Press Ltd., New York) 3rd Ed. 1357-1365 (1994)).

Choudhury *et al.* (J. Biol. Chem. 272(20):13446-13451 (1997)) report on a secretion-incompetent variant null of α-antitrypsin designated as Hong Kong.

**E. Overview of the Invention.**

The current invention is based on the unexpected discovery that inhibition of UGGT or other elements of the ER-chaperon retention machinery allows mis-folded or mis-assembled proteins, such as mis-folded mutant (ΔF508) CFTR protein and mutant α-antitrypsin (Hong Kong), to exit the ER instead of being targeted for degradation. By preventing the normal action of UGGT and/or other elements of the ER-chaperon retention machinery, the mis-folded proteins exit the ER and are targeted to the plasma membrane,